Research Article

Aminated β-Cyclodextrin-Modified-Carboxylated Magnetic Cobalt/Nanocellulose Composite for Tumor-Targeted Gene Delivery

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Gene therapy is a new kind of medicine, which uses genes as drugs in order to treat life threatening diseases. In the present work, a nonviral vector, aminated β-cyclodextrin-modified-carboxylated magnetic cobalt/nanocellulose composite (ACDC-Co/NCC), was synthesized for efficient transfection of genes into tumour cells. The synthesized ACDC-Co/NCC was characterized by means of FTIR, XRD, SEM, and ESR techniques. DNA condensing ability of ACDC-Co/NCC was found to be increased with increase in amount of ACDC-Co/NCC and 84.9% of DNA ($1.0\,\mu$g/mL) inclusion was observed with $6.0\,\mu$g/mL of ACDC-Co/NCC. The cytotoxicity of ACDC-Co/NCC was observed to be minimal, even at higher concentration, with respect to the model transfecting agent, poly(ethyleneimine) (PEI). 88.2% of the gene was transfected at high dose of DNA, as indicated by the highest luciferase expression. These results indicated that ACDC-Co/NCC might be a promising candidate for gene delivery with the characteristics of good biocompatibility, potential biodegradability, minimal cytotoxicity, and relatively high gene transfection efficiency.

1. Introduction

Gene delivery, the incorporation of foreign DNA particles into the host cells, is considered as one of the major objectives in the development of nucleic acid therapeutics. Gene therapy is a suitable substitute for conventional protein therapy because the problems such as bioavailability, systemic toxicity, manufacturing cost, and *in vivo* clearance rate can be solved [1]. It offers the opportunities and potential to cure both genetic and acquired diseases, such as hemophilia, cystic fibrosis, and cancers [2]. Previous experiments have confirmed that, at the molecular level, cancer is due to defects in the cellular DNA. The molecular pathogenesis of human cancer is due to structural and/or functional alterations of specific genes whose normal function is to control cell birth and cell death [3].

Gene delivery can be mediated by viral and nonviral methods. In viral methods, viruses deliver genetic materials to the specific disease sites via their efficient inherent mechanism. Viral vectors can provide efficient transduction and high gene expression. However, the use of viral vectors is quite limited due to safety concerns such as production of immunogenic reactions or mutagenesis of transfected cells [4]. These have necessitated the design of nonviral vectors as potential alternative which produces less complexity.

Nonviral vectors are attractive gene delivery systems for tumour gene therapy [5]. These have low immunogenicity, are easier to manufacture, have no signs of oncogenicity, and are relatively cheap compared to viral vectors [6]. In the past decade, a number of nonviral vectors such as cationic polymers (polyplexes), dendrimers, chitosans, liposomes, or cyclodextrins have been widely used in gene therapy [7–11]. Cyclodextrins (CDs) are cyclic α-(1,4-) linked oligosaccharides of α-D-glucopyranose and have been extensively used as carriers to improve the solubility, stability, and bioavailability of chemical drugs, due to their low toxicity, lack of immunogenicity, biocompatibility, and ability to form inclusion complexes with a variety of guest molecules in solution and solid state [12]. β-CD is the most commonly used CD in pharmaceutical formulations and has been extensively studied in

human [6]. Many authors have used CD as an efficient carrier for gene transfection [13]. The formation of inclusion complex is not fixed and the interaction is highly favorable as it reduces the ring strain leading to a more stable lower energy state [14].

Using magnetic nanoparticles to enhance gene transfection, a recently developed technique termed magnetofection has been shown to be a powerful technology in gene delivery [15]. In this method, the complexes of nucleic acids and their vectors are combined with magnetic nanoparticles, so they can be drawn and concentrated to the target cells by applying a magnetic field. In addition to facilitating targeted gene delivery, the rapid sedimentation of the gene-particle complex onto the target area significantly reduces both the time and dose of the vector to achieve efficient transfection [16]. Magnetofection was found to be less cytotoxic than standard transfection. While searching for suitable elements for the magnetic core, transition metals like Fe, Ni, Co, and Mn were found to be good candidates since they show high magnetization values which is important for high performance MRI and hyperthermia applications [17]. Magnetic hyperthermia, a thermal treatment of cancerous cells based on the fact that the cancer cells are more susceptible to high temperatures than the healthy ones, is regarded as a complementary technique to chemo- and radiotherapy as increasing the effects of these therapies and the MNPs can be used as heating mediators. In hyperthermia application, Co, being a hard magnetic material, is doped to other ferrites (MFe_2O_4) in changing concentrations in order to increase the magnetic anisotropy eventually to improve the heat transfer rate.

The coating of magnetic core materials prevents the leaching of potentially toxic components into the body during *in vivo* applications. Coating of magnetic nanoparticles with natural polymers such as carbohydrates and proteins is common [18, 19], among which carbohydrates are particularly popular owing to their biocompatibility. Moreover, functionalization is possible by making use of the hydroxyl groups on the carbohydrate skeletons [20]. Nanocellulose (NC) is an attractive biotemplate material for the fabrication of nanostructures. This NC has been used widely as stabilizing matrix for the metal nanoparticles and the developed composites have been used in various biomedical applications, such as antimicrobial wound dressing [21, 22]. The inclusion of metallic nanoparticles can be achieved in the NC matrix, by the treatment of metallic salts with reducing agents (e.g., $NaBH_4$) [23].

The airway and alveolar epithelium are the sites where genetic disorders such as cystic fibrosis or inherited surfactant protein B deficiency and lung cancer have major fatal impacts. Hence, the respiratory system has been considered as an attractive target for gene therapy intervention, in addition to its large surface area and accessibility [24, 25]. Several authors have used magnetofection to transfect a number of cell types including primary lung epithelial cells [26] and blood vessel endothelial cells [27].

Given these premises, the aim of the present work was to develop a novel nonviral gene vector, aminated β-cyclodextrin-modified-carboxylated magnetic cobalt/nanocellulose composite (ACDC-Co/NCC), with improved transfection efficiency to epithelial cells. Magnetofection may be effective for targets close to the body's surface (epithelial cells), as the magnetic field strength falls off rapidly with distance [28]. β-CD has the merit of a hydrophobic cavity which is easy to assemble with genes. The grafting of CD molecules onto the magnetic cobalt nanoparticles/nanocellulose matrix may lead to a molecular carrier that possess the cumulative effects of inclusion, size specificity, and transport properties of CD and the site specificity of Co nanoparticles to target cells by applying a magnetic field, commonly called "magnetofection." Triethylenetetramine (TETA) has large number of positive sites or secondary amine groups which will easily condense the negatively charged genes to form polyplexes [29]. Moreover, the positive charge of TETA is highly desirable for effective cell transfection as cell membranes are negatively charged. Effective gene delivery depends on its stability and ability to transfect across the cell membrane and interferes with the host DNA. So, the systematic investigations on the formation of polymer/DNA complexes, gene transfection ability, and cell viability were evaluated in alveolar epithelial cells (A549 cell line).

2. Experimental

2.1. Materials. Nanocellulose crystals were extracted from the sawdust of *Mangifera indica*, collected from local sawmill, Trivandrum. Cobalt chloride hexahydrate (M_W: 129.84 g/mol) is obtained from SD Fine-Chem Ltd. (Mumbai, India). The plasmid pCMVLuc (VR-1216) encoding Luciferase (Clontech, Palo, Alto, CA, USA) was used for carrying out the transfection experiments. Luciferase activity assay kit and protein assay kit were obtained from Promega (Madison, USA) and Bio-Rad Labs (Hercules, USA), respectively. Ethidium bromide (EtBr), 2,2,6,6-tetramethyl-1-(piperidinyloxy) radical (TEMPO), TETA, triethylamine (TEA), and β-CD (M_W: 1135 g/mol) were obtained from Sigma-Aldrich, Milwaukee, WI, USA. Sodium borohydride ($NaBH_4$), H_2O_2, sulfuric acid, sodium hydroxide, NH_4OH, $NaClO$, $NaBr$, $NaHCO_3$, p-toluenesulfonyl chloride, acetonitrile, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), and HCl were purchased from E-Merck (Worli, Mumbai, India) and used without further purification. Distilled water was used throughout the study.

2.2. Preparation of ACDC-Co/NCC. The synthesis of ACDC-Co/NCC involves the following steps, as demonstrated in Figure 1.

2.2.1. Extraction of Nanocellulose from Sawdust. The synthesis of nanocellulose was done following the procedure as already reported in our previous literature [30]. Briefly, sawdust was pretreated with 10% H_2SO_4 solution (120°C, 10 min) and centrifuged to remove rich pentosans solution. Delignification was achieved by the subsequent treatment with 1% NaOH (100°C, 1 h). The obtained brown mass was bleached with 5% H_2O_2 (80°C, 1 h) yielded white cellulose as the product [31]. About 5 g of the extracted cellulose was dispersed in 250 mL distilled water under magnetic stirring (20 min). 140 mL 98%
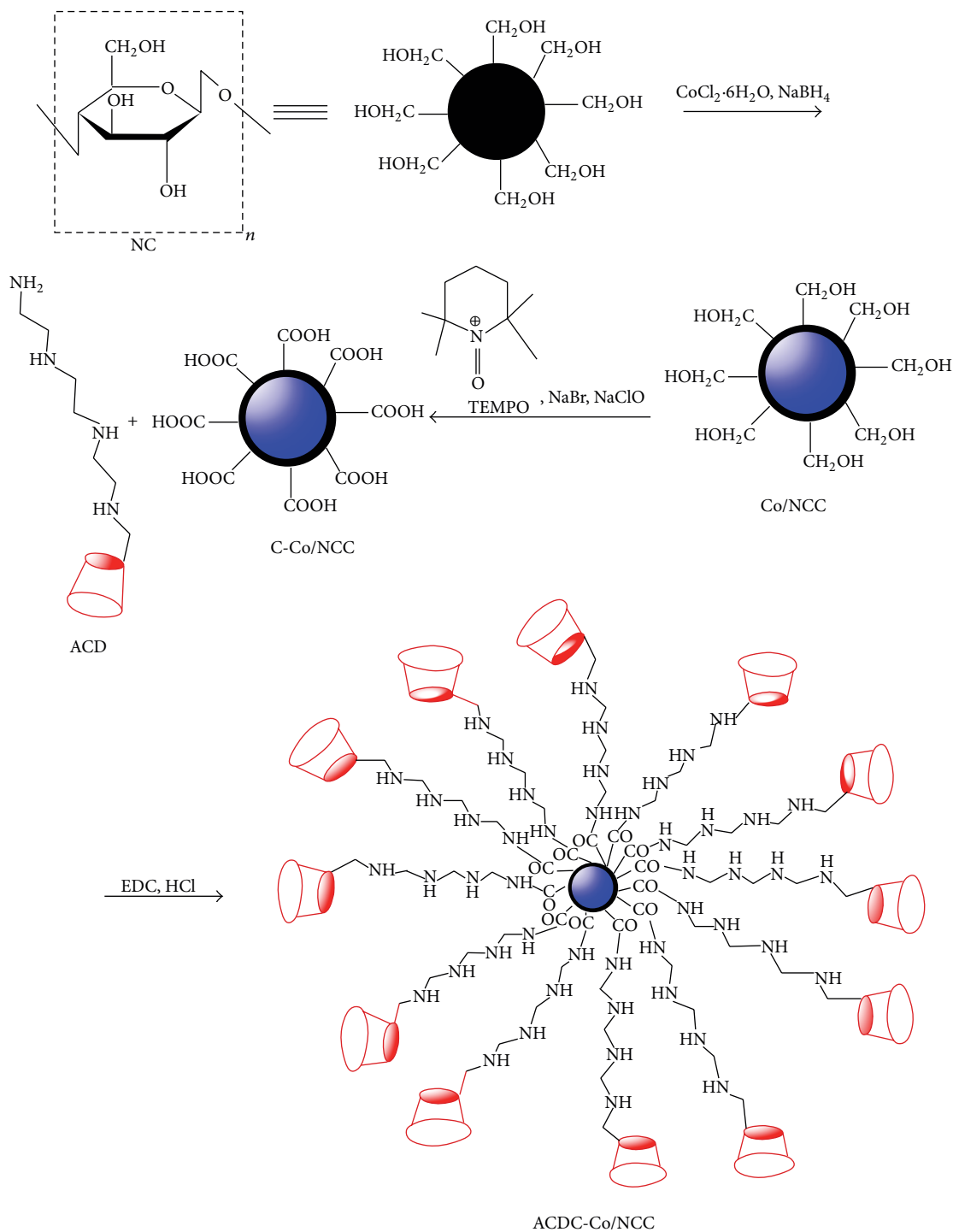
FIGURE 1: Predicted mechanism for the synthesis of the gene carrier, ACDC-Co/NCC.

sulfuric acid was dropped to the homogenized mixture, without causing heating. After complete addition, the mixture was heated at 50°C for 2 h. The hot mixture was diluted ten times with ice cooled distilled water. The obtained white colloid was centrifuged, washed many times with water, and freeze-dried [32].

2.2.2. Synthesis of Co/NCC. The nanocomposite of Co and NC was prepared via chemical precipitation method. Diffusion of Co^{2+} into the nanocellulose matrix was carried out under intensive stirring of 1 g of NC in 20 mL 1 M NH_4OH and 2 mL 0.1 M of $CoCl_2\cdot6H_2O$ solution for 1 h at 25°C and pH value of 12. To the obtained suspension, we added 20 mL

solution of 0.30 M NaBH$_4$ and stirred intensively for 1 h at 50°C.

2.2.3. Preparation of Carboxylated Co/NCC (C-Co/NCC).
Nanocellulose was carboxylated according to the procedure by Araki et al. [33], as follows. 5.0 g NC was suspended in 500 mL distilled water, to which 0.5 g of TEMPO and 5.0 g of NaBr were added. We added NaClO solution (2.0 mL) and the mixture was stirred for 4 h at room temperature with the pH adjusted to 10-11 by 3 N NaOH. The obtained carboxylated magnetic nanocrystals were collected by the addition of ~30 g of NaCl and 0.1 N HCl and finally dialyzed against deionized water.

2.2.4. Preparation of Tosyl CD (TCD).
Mono-6-deoxy-6-(p-toluenesulfonyl)-CD (TCD) was synthesized [34] as discussed below. β-CD (20 g, 16.3 mmol) was suspended in 150 mL of water, and NaOH (2.16 g, 54.7 mmol) in 7 mL of water was added dropwise for 6 min. The suspension became homogeneous and slightly yellow before the addition was complete. p-Toluenesulfonyl chloride (3.36 g, 17.6 mmol) in 10 mL of acetonitrile was added dropwise over 8 min, causing immediate formation of a white precipitate. After 2 h of stirring at room temperature, the precipitate was removed by filtration and cooled overnight at 4°C.

2.2.5. Preparation of Aminated CD (ACD).
ACD was prepared via the reaction of TCD with TETA [35]. Briefly, TETA (0.8 g) and TCD (4.0 g) were dissolved in water (30 mL) containing TEA (20 mL), and the stirred mixture was heated to reflux under nitrogen atmosphere for 24 h. After evaporation under reduced pressure, the residue was poured into the vigorously stirred anhydrous ethanol (500 mL), and the resultant mixture was filtered, washed, and freeze-dried to obtain a slight yellow precipitate.

2.2.6. Preparation of ACDC-Co/NCC.
The gene carrier, ACDC-Co/NCC, was prepared using the procedure, as follows [36]. About 0.085 g (0.40 mmol) of the C-Co/NCC and 0.567 g (0.50 mmol) of the ACD were stirred in 3 mL water for 1 h. 1 mL of EDC solution (0.52 mmol, 10%) was added to the reaction mixture and was stirred for 48 h. The pH of the solution was adjusted to 2.0 by the addition of HCl and the solution was dialyzed against distilled water for 5 days. The obtained product was freeze-dried.

2.3. Estimation of Carboxyl Groups.
Boehm titration was performed to determine the amount of free carboxyl (–COOH) groups. Briefly, about 0.15 g of powdered C-Co/NCC was shaken with 30 mL NaHCO$_3$ for 20 h and filtered. An aliquot of the filtrate was mixed with excess of 0.05 M HCl and the CO$_2$ gas formed was boiled off and back titrated with 0.05 M NaOH [37]. The amount of carboxylic acid groups was estimated using the equation:

$$\text{COOH (meq/g)} = \frac{(C_{\text{NaOH}} \times V_{\text{NaOH}} - C_{\text{HCl}} \times V_{\text{HCl}})}{W}, \quad (1)$$

where C is the concentration of HCl or NaOH (M), W is the weight of the C-Co/NCC (g), and V is the volume of HCl or NaOH solution used (mL).

2.4. DNA Binding Studies.
High DNA condensing ability is an important factor for a successful polymeric gene delivery system [38]. Here the inclusion of DNA in the hydrophobic cavity of the CD in the ACDC-Co/NCC was carried out as follows. The polymeric material, ACDC-Co/NCC, and DNA plasmid solution (in PBS buffer) were mixed in equivalent medium. The mixture was vortexed and then stayed at ambient conditions for 30 min before use. The DNA condensing abilities of ACDC-Co/NCC were measured using ethidium bromide (EtBr) assay. Briefly, EtBr (153 μL of a 0.01% solution) was added to 96 μg of DNA and diluted to 6 mL. Desired concentrations of ACDC-Co/NCC were added to each well of a 96-well microplate, respectively, and 50 μL of the DNA-EtBr solution was added to obtain a final volume of 100 μL. After incubation for 5 min, the complexes were treated with 100 μL of distilled water. The fluorescence was measured using HITACHI F-7000 fluorescence spectrophotometer. The λ_{ex} and λ_{em} were set at 526 nm and 592 nm, respectively, as they are characteristic of DNA. Decrease in fluorescence intensity would result in higher inclusion % due to the unavailability of DNA to ethidium bromide. The inclusion % of DNA in the cyclodextrins was calculated using the formula:

$$\text{inclusion \%}$$
$$= \left[1 - \frac{\text{Concentration of DNA measured by fluoresence}}{\text{Practical concentration of DNA}} \right]$$
$$\times 100. \quad (2)$$

2.5. MTT Cytotoxicity Assay.
The cytotoxicity or cell viability was evaluated by MTT assay using A549 cell (adenocarcinomic human alveolar basal epithelial cell line). Briefly, A549 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum and 100 units/mL of penicillin/streptomycin at 37°C in a humidified atmosphere (5% CO$_2$/95% O$_2$). The cells were seeded into each well of a 96-well plate (10^4 cells/well). After 24 h, the ACDC-Co/NCC complexes were prepared in culture medium and the media in the wells were replaced with the 200 μL culture medium-sample mixture (equal to 1 μg DNA). The plates were placed in an incubator and allowed to attach overnight. After incubation, culture medium and 20 μL of MTT (3-(4,5-dimethylthiazol-2)-2,5-diphenyltetrazolium bromide) solution were used to replace the mixture in each well and incubated for further 4 h in 5% CO$_2$ at 37°C. Then the culture medium and MTT were removed. The developed purple colored formazan crystals were dissolved in DMSO and analyzed by measuring absorbance at 570 nm in a colorimeter. The quantity of formazan crystals is directly proportional to the number of live cells. Cells incubated with pure media without treatment were considered as controls and the cell viability values were set as 100%. For comparing the cell viability, the widely used transfecting agents such

as PEI and its complex with DNA were considered, after following the cytotoxicity assay procedure. The cell viability (%) was calculated according to the following equation:

$$\text{Cell viability} \ (\%) = \frac{A_{\text{Sample}} - A_{\text{Blank}}}{A_{\text{Control}} - A_{\text{Blank}}}, \tag{3}$$

where A_{Sample} and A_{Control} represent absorption from the cells treated with the polymer complexes and A_{Blank} represented absorption from the cells not treated with MTT.

2.6. In Vitro Gene Transfection and Gene Expression Experiments. The DNA transfection efficiency of ACDC-Co/NCC *in vitro* was evaluated using A549 cell lines following the procedure, reported earlier. Briefly, cells were separately seeded at a density of 10^4 cells/well in a 96-well plate in 500 mL DMEM medium containing 10% foetal bovine serum (FBS) and incubated for 18 h before transfection. The medium was replaced with 400 mL fresh serum-free DMEM containing complexes with 1 mg DNA. After incubation for 4 h at 37°C, the transfection medium was replaced with fresh growth medium and then the cells were incubated for an additional 48 h.

Luciferase activity was measured according to standard protocols of luciferase assay system. The total protein was measured according to a BCA protein assay kit (Pierce) and transfection efficiency was reported in terms of luciferase activity.

3. Results and Discussion

3.1. Gene Carrier-Preparation and Properties. The preparation of the gene carrier involves six steps, as described in the Experimental section. At first, cellulose was extracted from the value added product, sawdust, by acid-alkali treatment followed by bleaching with H_2O_2. Sawdust basically contains cellulose, lignin, and polyoses. The treatment of sawdust with H_2SO_4 (10%) and NaOH (1%) removed the polyoses and lignin, respectively. The obtained brown mass when bleached with 5% H_2O_2 yielded a white cellulose powder. This cellulose powder was made into nanocrystals via acid hydrolysis which improves its crystallinity and hydrophilicity and it looks like a white pasty material. Since NC occupies a large number of hydroxyl groups, its swelling capacity would be high, and this property can be exploited for the production of biocompatible materials.

The Co/NCC prepared via chemical precipitation method appears like a flesh colored precipitate, where Co^{2+} ions get encapsulated in the NC matrix. The surface coating ensures a double-sided isolation both preventing the release of toxic cobalt ions from magnetic core into the biological media and shielding the magnetic core from oxidization and corrosion. The surface coating molecules such as antibodies, peptides, and polysaccharides permit specific recognition of cell types and target the nanoparticles to a specific tissue or cell type by binding to a cell surface receptor. Even though Co is a highly toxic metal when compared to the commonly used Fe, it is reported that it can be successfully utilized for *in vivo* applications after proper encapsulation. During *in vivo* applications once the magnetic nanoparticles (MNPs) are injected into the body, they are recognized by the body's major defence system, which eliminates any foreign substance from blood stream. However, the specific surface coatings elongate the MNPs circulation time in blood and maximize the possibility to reach target tissues [17].

The Co/NCC was carboxylated using TEMPO where the primary hydroxyl groups in the nanocellulose were converted to carboxyl groups. The amount of carboxyl groups in the C-Co/NCC was estimated to be 2.23 meq g^{-1}. The gene carrier, ACDC-Co/NCC, was prepared by the amidification reaction between C-Co/NCC and aminated cyclodextrin (ACD) using EDC. EDC forms an unstable intermediate complex with carboxylic groups, which is susceptible to amide bond formation if it reacts with a primary amine. The yield of ACDC-Co/NCC was found to be 68.6%. The long chain in the ACDC-Co/NCC arises from TETA, increases the flexibility to capture the DNA molecules, and helps in their condensation owing to large amount of amino groups in the chain.

3.2. Characterization Studies

3.2.1. FTIR Spectra. Figure 2 presents the FTIR spectra of the cellulose, NC, Co/NCC, C-Co/NCC, and ACDC-Co/NCC. The IR spectra of cellulose show the characteristic broad peak related to hydrogen bonded O–H stretching vibration at 3441 cm^{-1} and the peak at 2922 and 1033 cm^{-1} could be attributed to the C–H stretching and C–H bending of the CH$_2$ group. In 1636 and 668 cm^{-1}, there are signals corresponding to the C=O stretching of hemicelluloses and β-glycosidic linkage in cellulose, respectively. The band at 897 cm^{-1} is characteristic of glycosidic ring in cellulose structure. NC shows all the characteristic peaks of cellulose and the position may somehow change due to the breaking of intermolecular hydrogen bonding between the cellulose chains. The absorption band at 607 cm^{-1} in Co/NCC is associated with the metal–O stretching vibration which confirms the presence of Co nanoparticles. The C-Co/NCC shows a new sharp adsorption peak at 1729 cm^{-1}, a characteristic of an ester carbonyl group. The absorption at 1391 cm^{-1} in ACDC-Co/NCC is attributed to the in-plane bending vibrations of hydroxy groups, which may arise from large number of cyclodextrin molecules. Meanwhile, the asymmetrical and symmetrical stretchings of methylene (–CH$_2$–) appear at 2919 and 2853 cm^{-1}, and the in-plane bending of CH$_2$ is located at about 1462 cm^{-1}. In addition, the new band at 1056 cm^{-1} is responsible for the stretching vibration of C–O. The presence of peaks at the vibration frequencies, 1646 cm^{-1} (C=O stretch), 1554 cm^{-1} (amide linkage), and 1407 cm^{-1} (primary amides), also confirms the functionalization onto ACDC-Co/NCC.

3.2.2. XRD Analysis. The XRD patterns of cellulose, NC, Co/NCC, and ACDC-Co/NCC are shown in Figure 3. In the XRD pattern of cellulose, the diffraction maxima at $2\theta =$ 5.8, 22.1, and 34.2° can be attributed to the partial crystalline nature of cellulose-like natural polymers [39]. The characteristic peaks of NC are the same as that of cellulose; however, its broadness increased to a small extent which indicates
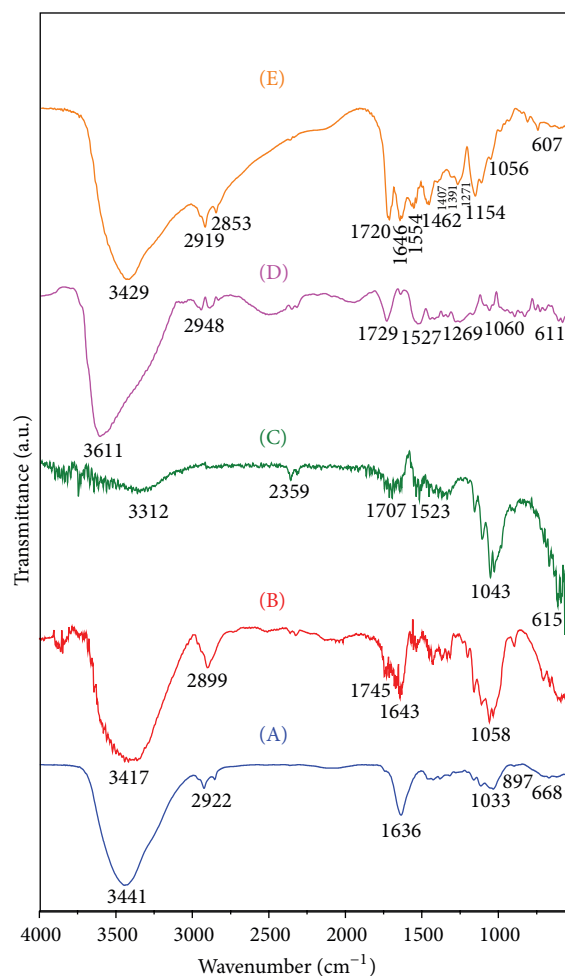
FIGURE 2: FTIR spectra of (A) Cellulose, (B) NC, (C) Co/NCC, (D) C-Co/NCC, and (E) ACDC-Co/NCC.

FIGURE 3: XRD patterns of (A) Cellulose, (B) NC, (C) Co/NCC, and (D) ACDC-Co/NCC.

the reduced particle size. The sharper diffraction peaks are indication of a high degree of crystallinity in the structure, which arises due to the partial removal of the amorphous regions during the acid hydrolysis treatment of cellulose [40]. The percentage crystallinity of cellulose and NC was compared using the equation [41]:

$$\text{Crystallinity } (\%) = \frac{(I_c - I_a)}{I_c} \times 100, \quad (4)$$

where I_c and I_a are the intensity of the crystalline and amorphous peaks, respectively. The percentage crystallinity of cellulose and NC, using this equation, was 67.05% and 81.74%, respectively. The high percentage crystallinity of NC as compared to cellulose may be due to the endoglucanase mediated hydrolysis during their preparation.

The average crystallite size of NC and Co/NCC calculated by fitting Lorentzian and using Debye-Scherrer formula [42] was 21.6 and 19.2 nm, respectively. The shifting of characteristic peaks of cellulose in Co/NCC suggests the presence of dopant in the matrix. The increased amorphous nature and disappearance of characteristic peaks in diffraction pattern
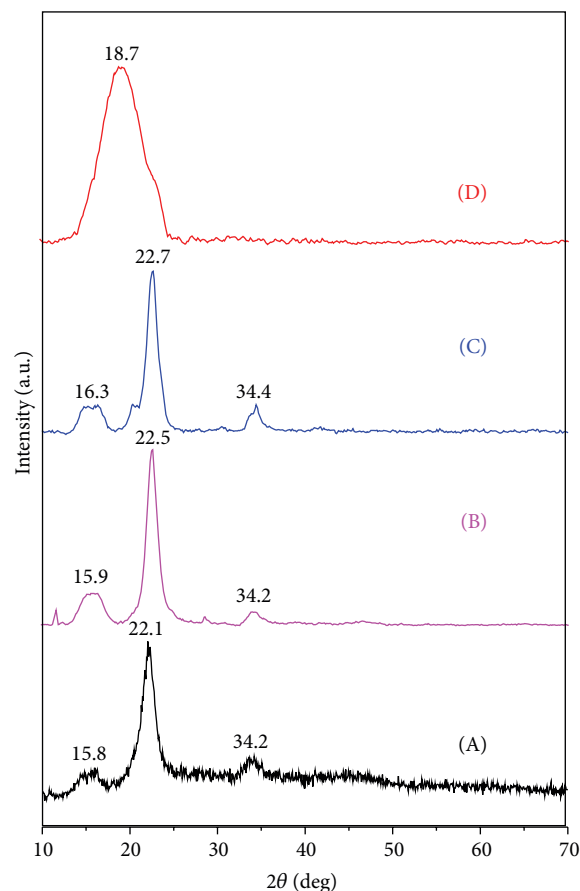
of ACDC-Co/NCC indicate the modification of the surface of Co/NCC.

3.2.3. SEM Analysis. Figure 4 shows the surface morphology of (a) cellulose, (b) NC, (c) Co/NCC, and (d) ACDC-Co/NCC. As seen in Figure 4(a), cellulose has smooth, fibrous, and layered structure, while NC (Figure 4(b)) possesses spindle structure. NC is hydrophilic and behaves like a gel structure. This hydrophilicity of NC may increase the swelling ability and diffusion of solute molecules from aqueous phase. However, Co/NCC shows distinct small gel structures, and these composites are suitable for magneto-fection. Also, Co/NCC is irregular, uneven, and rough. The increase in size and swellable structure of ACDC-Co/NCC clearly indicates proper grafting and linking of cyclodextrin molecules to the polymeric chain. The surface of ACDC-Co/NCC appears to consist of large number of soft folds. Moreover, the folding will provide more surface area for the intake of DNA.

3.2.4. ESR Spectra. ESR spectrum of ACDC-Co/NCC (Figure 5) clearly indicates the inclusion of cobalt magnetic nanoparticles within the polymer matrix. Since the polymer cannot be magnetized under this condition, the peak
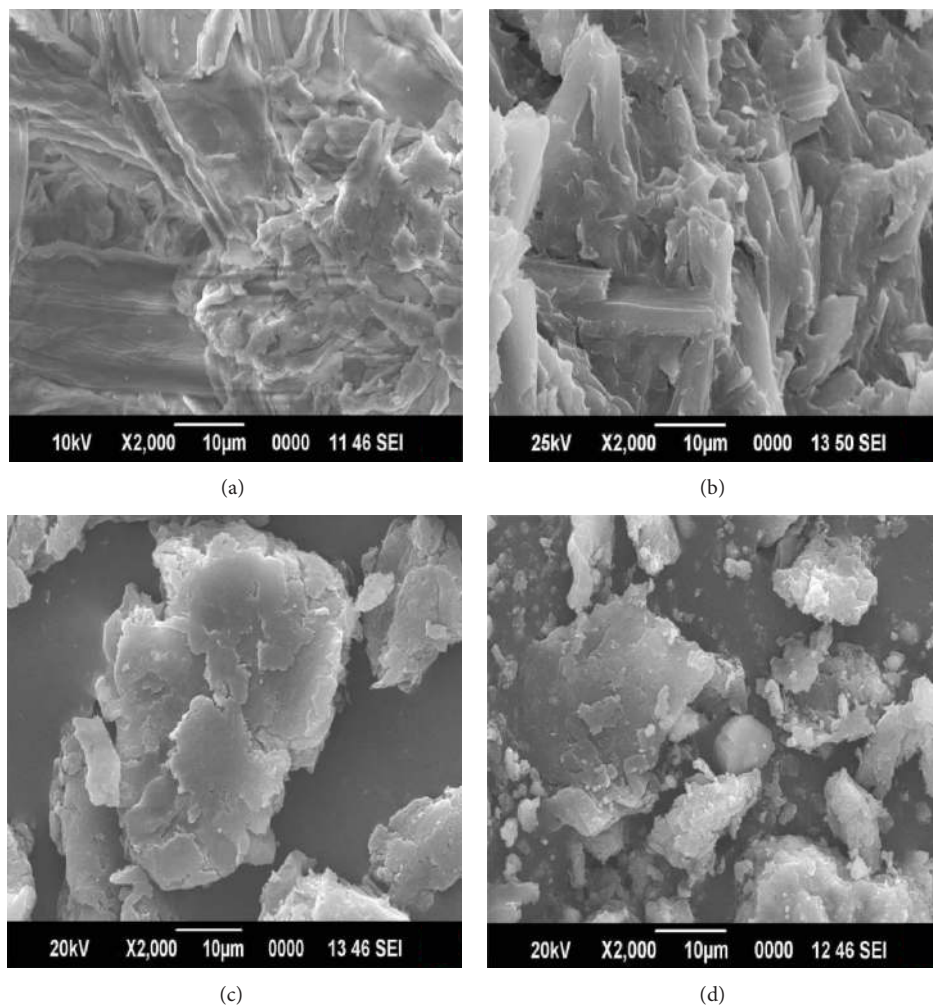
(a)

(b)

(c)

(d)

FIGURE 4: SEM photographs of (a) cellulose, (b) NC, (c) Co/NCC, and (d) ACDC-Co/NCC.

detected in the spectrum may correspond to the magnetic nanoparticles. The g-factor can be calculated from the ESR spectrum as

$$g = \frac{h\nu}{\beta H_r}, \tag{5}$$

where h is the Planck constant (6.63×10^{-27} ergs), β is the universal constant (9.27×10^{-21} erg/Gs), ν is the frequency (9.1×10^9 Hz), and H_r is the resonance of magnetic field applied (Gs). Here, the g-factor was found to be 2.01 for ACDC-Co/NCC and the magnetic field (H_r) 3230 Gs was found sufficient to excite all of the dipole moments present in 1.0 g of the ACDC-Co/NCC. The H_r value was changed from 1000 Gs to 20,000 kGs for various applications [43], which implies that the present gene carrier needs less magnetic intensity to target the desired cells using a conventional permanent magnet.

3.3. DNA Inclusion Studies. The inclusion of DNA to the pocket of β-CD was found to be increased from 19.7 to 84.9% with increase in the concentration of ACDC-Co/NCC from 1.0 to 6.0 μg/well (Figure 6). This is due to the large availability

of cyclodextrins which can form inclusion complexes with DNA. In addition to this, the TETA can condense the DNA to form complexes through electrostatic interaction between its protonated amine groups and the negatively charged phosphodiester groups of the DNA. However, the formulations loaded with higher amount of ACDC-Co/NCC (>6.0 μg/well) exhibited only negligible % of inclusion.

3.4. The Gene Transfection. The *in vitro* gene delivery efficiency of the complexes was first evaluated using luciferase at various DNA concentrations. The gene transfection studies (Figure 7) results indicated that the ACDC-Co/NCC exhibited significant transfection performance across the A549 cell line. 88.2% of the gene was observed to be transfected at high dosage of DNA (1000 ng DNA/well). More than 70% of the DNA transfection efficiency was achieved even at low dosage of DNA (50 ng DNA/well), indicating the outstanding gene delivery ability of ACDC-Co/NCC. Compared to the leading gene transfecting agent PEI, the ACDC-Co/NCC exhibited significantly improved gene transfection efficiency in A549 cell line. Thus, for the A549 cell line, ACDC-Co/NCC showed
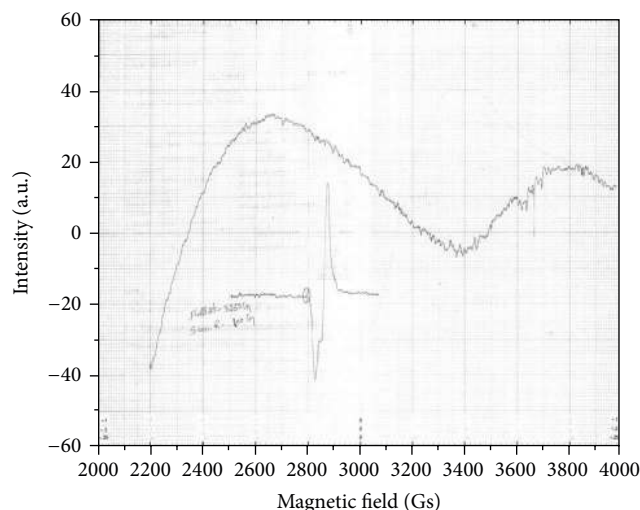
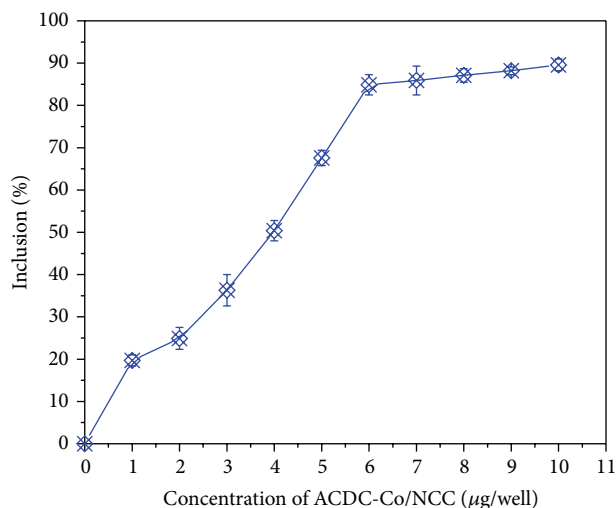FIGURE 5: ESR spectrum of ACDC-Co/NCC.

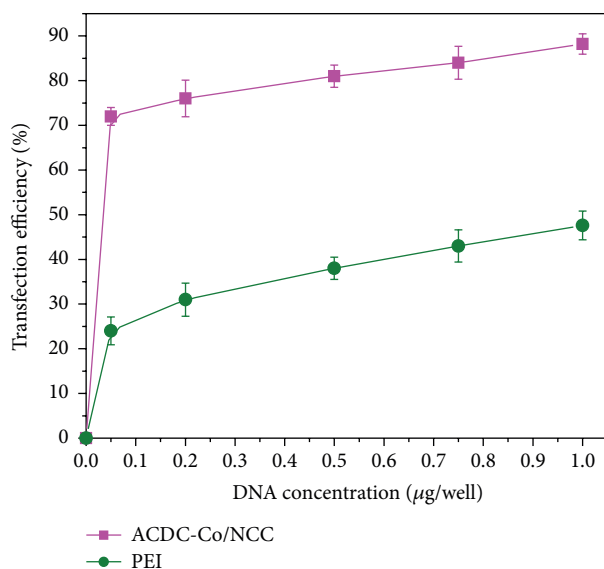FIGURE 6: Percentage inclusion of DNA with respect to the concentration of ACDC-Co/NCC.

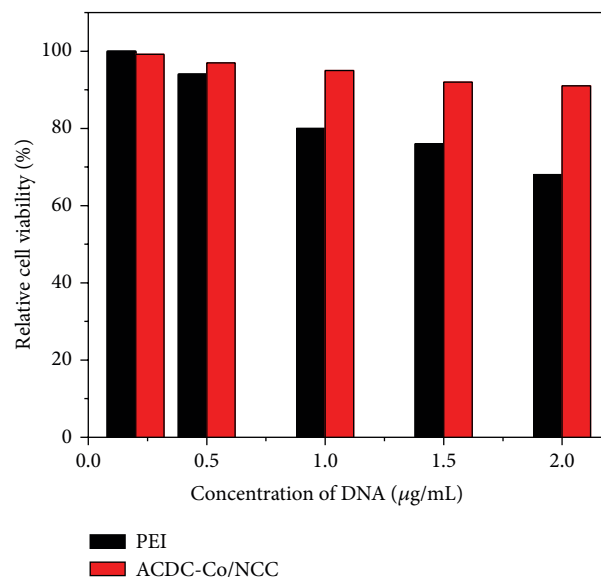FIGURE 7: *In vitro* luciferase expression in A549 cancer cells.

FIGURE 8: Relative cell viability of PEI and ACDC-Co/NCC.

higher transfection efficiency and exhibited the highest gene expression.

3.5. Cell Viability. The use of polymeric gene vectors for the safe clinical gene therapy depends on their cytotoxicity. The cytotoxicity of polymer/DNA complexes not only leads to apoptotic cell death but also is associated with the attenuation of gene expression. Figure 8 shows the *in vitro* MTT assay results of cytotoxicity of the ACDC-Co/NCC and PEI complexes in A549 cancer cells. Cell viability assay results indicated that both the polymer complexes with DNA exhibited minimal toxicity, with the cell cultures maintaining high viability. Among the polymers, ACDC-Co/NCC demonstrated a significantly lower toxicity than that of PEI. It was observed that PEI has a faster increment in toxicity with increase in concentration. Several authors observed this phenomenon during the gene transfection studies [44, 45]. More than 95% of DNA transfected cells showed normal viability even in the presence of high dosage of reagent (1000 ng DNA/well). Thus, our novel gene carrier, ACDC-Co/NCC, not only reduced the toxicity but also increased the transgene expression level as well, indicating that ACDC-Co/NCC could be a promising agent for gene delivery.

4. Conclusions

The safe and efficient delivery of genes into cells is one of the major objectives in the development of nucleic acid therapeutics. The aim of the present work was to develop a novel gene carrier, aminated β-cyclodextrin-modified-carboxylated magnetic cobalt/nanocellulose composites (ACDC-Co/NCC), for the efficient transfection of genes into tumour cells. The gene carrier was analyzed using FTIR, XRD, SEM, and ESR spectral techniques. DNA condensing ability of ACDC-Co/NCC was measured using ethidium bromide

assay and was found to be increased with increase in ACDC-Co/NCC. The cytotoxicity of ACDC-Co/NCC was observed to be minimal, even at higher concentration, with respect to the model transfecting agent, PEI. 88.2% of the gene was transfected at high dose of DNA, as indicated by highest luciferase expression. These results suggest that ACDC-Co/NCC can be used as a reliable carrier for gene delivery.

Conflict of Interests

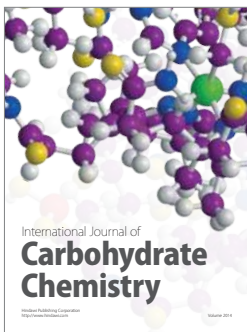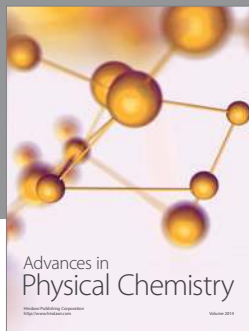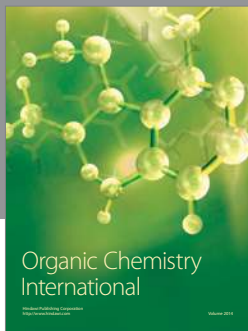The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

References

[1] S. Y. Wong, J. M. Pelet, and D. Putnam, "Polymer systems for gene delivery-past, present, and future," *Progress in Polymer Science*, vol. 32, no. 8-9, pp. 799–837, 2007.

[2] T. J. Anchordoquy, S. D. Allison, M. D. C. Molina, L. G. Girouard, and T. K. Carson, "Physical stabilization of DNA-based therapeutics," *Drug Discovery Today*, vol. 6, no. 9, pp. 463–470, 2001.

[3] R. Bernards and R. A. Weinberg, "A progression puzzle," *Nature*, vol. 418, no. 6900, p. 823, 2002.

[4] K. Pal, A. K. Banthia, and D. K. Majumdar, "Polymeric hydrogels: characterization and biomedical applications—a mini review," *Designed Monomers and Polymers*, vol. 12, no. 3, pp. 197–220, 2009.

[5] C. Zhang, S. Gao, W. Jiang et al., "Targeted minicircle DNA delivery using folate-poly(ethylene glycol)-polyethylenimine as non-viral vector," *Biomaterials*, vol. 31, pp. 6075–6086, 2010.

[6] M. Eng and A. Elkordy, "Gene delivery using non-viral vectors (cyclodextrins) with Pluronic-F127 and folic acid," in *Proceedings of the 2nd Electronic Conference on Pharmaceutical Sciences*, Sciforum Electronic Conferences Series, pp. 1–39, 2012.

[7] J. S. Choi and J. S. Park, "Design elements of polymeric gene carrier," in *Biomaterials for Delivery and Targeting of Proteins and Nucleic Acids*, R. Mahato, Ed., CRC Press, Boca Raton, Fla, USA, 2005.

[8] L. De Laporte, J. Cruz Rea, and L. D. Shea, "Design of modular non-viral gene therapy vectors," *Biomaterials*, vol. 27, no. 7, pp. 947–954, 2006.

[9] H. Lv, S. Zhang, B. Wang, S. Cui, and J. Yan, "Toxicity of cationic lipids and cationic polymers in gene delivery," *Journal of Controlled Release*, vol. 114, no. 1, pp. 100–109, 2006.

[10] D. Putnam, "Polymers for gene delivery across length scales," *Nature Materials*, vol. 5, no. 6, pp. 439–451, 2006.

[11] M. A. Mintzer and E. E. Simanek, "Nonviral vectors for gene delivery," *Chemical Reviews*, vol. 109, no. 2, pp. 259–302, 2009.

[12] G. P. Tang, H. Y. Guo, F. Alexis et al., "Low molecular weight polyethylenimines linked by β-cyclodextrin for gene transfer into the nervous system," *Journal of Gene Medicine*, vol. 8, no. 6, pp. 736–744, 2006.

[13] V. Burckbuchler, V. Wintgens, C. Leborgne et al., "Development and characterization of new cyclodextrin polymer-based DNA delivery systems," *Bioconjugate Chemistry*, vol. 19, no. 12, pp. 2311–2320, 2008.

[14] A. Rasheed, A. Kumar, and V. V. N. S. S. Sravanthi, "Cyclodextrins as drug carrier molecule: a review," *Scientia Pharmaceutica*, vol. 76, no. 4, pp. 567–598, 2008.

[15] S. C. McBain, H. H. P. Yiu, A. El Haj, and J. Dobson, "Polyethyleneimine functionalized iron oxide nanoparticles as agents for DNA delivery and transfection," *Journal of Materials Chemistry*, vol. 17, no. 24, pp. 2561–2565, 2007.

[16] F. Scherer, M. Anton, U. Schillinger et al., "Magnetofection: enhancing and targeting gene delivery by magnetic force in vitro and in vivo," *Gene Therapy*, vol. 9, no. 2, pp. 102–109, 2002.

[17] E. M. Umut, *Surface Modification of Nanoparticles Used in Biomedical Applications*, InTech, 2013.

[18] S. Liang, Y. Wang, C. Zhang et al., "Synthesis of amino-modified magnetite nanoparticles coated with Hepama-1 and radiolabeled with 188Re for bio-magnetically targeted radiotherapy," *Journal of Radioanalytical and Nuclear Chemistry*, vol. 269, no. 1, pp. 3–7, 2006.

[19] M. A. McDonald and K. L. Watkin, "Investigations into the physicochemical properties of dextran small particulate gadolinium oxide nanoparticles," *Academic Radiology*, vol. 13, no. 4, pp. 421–427, 2006.

[20] T. Heinze, T. Liebert, B. Heublein, and S. Hornig, "Functional polymers based on dextran," *Advances in Polymer Science*, vol. 205, no. 1, pp. 199–291, 2006.

[21] T. Maneerung, S. Tokura, and R. Rujiravanit, "Impregnation of silver nanoparticles into bacterial cellulose for antimicrobial wound dressing," *Carbohydrate Polymers*, vol. 72, no. 1, pp. 43–51, 2008.

[22] J. Cai, S. Kimura, M. Wada, and S. Kuga, "Nanoporous cellulose as metal nanoparticles support," *Biomacromolecules*, vol. 10, no. 1, pp. 87–94, 2009.

[23] E. Lam, K. B. Male, J. H. Chong, A. C. W. Leung, and J. H. T. Luong, "Applications of functionalized and nanoparticle-modified nanocrystalline cellulose," *Trends in Biotechnology*, vol. 30, no. 5, pp. 283–290, 2012.

[24] J. Vadolas, R. Williamson, and P. A. Ioannou, "Gene therapy for inherited lung disorders: an insight into pulmonary defence," *Pulmonary Pharmacology and Therapeutics*, vol. 15, no. 1, pp. 61–72, 2002.

[25] Z. Mohammadi, M. Abolhassani, F. A. Dorkoosh et al., "Preparation and evaluation of chitosan-DNA-FAP-B nanoparticles as a novel non-viral vector for gene delivery to the lung epithelial cells," *International Journal of Pharmaceutics*, vol. 409, no. 1-2, pp. 307–313, 2011.

[26] S. W. Gersting, U. Schillinger, J. Lausier et al., "Gene delivery to respiratory epithelial cells by magnetofection," *Journal of Gene Medicine*, vol. 6, no. 8, pp. 913–922, 2004.

[27] F. Krötz, H.-Y. Sohn, T. Gloe, C. Plank, and U. Pohl, "Magnetofection potentiates gene delivery to cultured endothelial cells," *Journal of Vascular Research*, vol. 40, no. 5, pp. 425–434, 2003.

[28] S. C. McBain, H. H. P. Yiu, and J. Dobson, "Magnetic nanoparticles for gene and drug delivery," *International Journal of Nanomedicine*, vol. 3, no. 2, pp. 169–180, 2008.

[29] B. Abdallah, A. Hassan, C. Benoist, D. Goula, J. P. Behr, and B. A. Demeneix, "A powerful nonviral vector for in vivo gene transfer

into the adult mammalian brain: polyethylenimine," *Human Gene Therapy*, vol. 7, no. 16, pp. 1947–1954, 1996.

[30] T. S. Anirudhan and S. R. Rejeena, "Adsorption and hydrolytic activity of trypsin on a carboxylate-functionalized cation exchanger prepared from nanocellulose," *Journal of Colloid and Interface Science*, vol. 381, no. 1, pp. 125–136, 2012.

[31] D. R. Mulinari and M. L. C. P. da Silva, "Adsorption of sulphate ions by modification of sugarcane bagasse cellulose," *Carbohydrate Polymers*, vol. 74, no. 3, pp. 617–620, 2008.

[32] J. R. Capadona, K. Shanmuganathan, S. Trittschuh, S. Seidel, S. J. Rowan, and C. Weder, "Polymer nanocomposites with nanowhiskers isolated from microcrystalline cellulose," *Biomacromolecules*, vol. 10, no. 4, pp. 712–716, 2009.

[33] J. Araki, M. Wada, and S. Kuga, "Steric stabilization of a cellulose microcrystal suspension by poly(ethylene glycol) grafting," *Langmuir*, vol. 17, no. 1, pp. 21–27, 2001.

[34] R. C. Petter, J. S. Salek, C. T. Sikorski, G. Kumaravel, and F.-T. Lin, "Cooperative binding by aggregated mono-6-(alkylamino)-β-cyclodextrins," *Journal of the American Chemical Society*, vol. 112, no. 10, pp. 3860–3868, 1990.

[35] X. Zhang, Z. Wu, X. Gao et al., "Chitosan bearing pendant cyclodextrin as a carrier for controlled protein release," *Carbohydrate Polymers*, vol. 77, no. 2, pp. 394–401, 2009.

[36] N. Aoki, M. Nishikawa, and K. Hattori, "Synthesis of chitosan derivatives bearing cyclodextrin and adsorption of *p*-nonylphenol and bisphenol A," *Carbohydrate Polymers*, vol. 52, no. 3, pp. 219–223, 2003.

[37] I. G. Shibi and T. S. Anirudhan, "Synthesis, characterization, and application as a mercury(II) sorbent of banana stalk (*Musa paradisiaca*)—polyacrylamide grafted copolymer bearing carboxyl groups," *Industrial and Engineering Chemistry Research*, vol. 41, no. 22, pp. 5341–5352, 2002.

[38] W.-F. Lai, G.-P. Tang, X. Wang et al., "Cyclodextrin-PEI-Tat polymer as a vector for plasmid DNA delivery to placenta mesenchymal stem cells," *BioNanoScience*, vol. 1, no. 3, pp. 89–96, 2011.

[39] L. V. A. Gurgel, O. K. Júnior, R. P. D. F. Gil, and L. F. Gil, "Adsorption of Cu(II), Cd(II), and Pb(II) from aqueous single metal solutions by cellulose and mercerized cellulose chemically modified with succinic anhydride," *Bioresource Technology*, vol. 99, no. 8, pp. 3077–3083, 2008.

[40] A. Alemdar and M. Sain, "Isolation and characterization of nanofibers from agricultural residues—wheat straw and soy hulls," *Bioresource Technology*, vol. 99, no. 6, pp. 1664–1671, 2008.

[41] K. Das, D. Ray, C. Banerjee et al., "Physicomechanical and thermal properties of jute-nanofiber-reinforced biocopolyester composites," *Industrial and Engineering Chemistry Research*, vol. 49, no. 6, pp. 2775–2782, 2010.

[42] P. Scherrer, "Bestimmung der Grösse und der inneren Struktur von Kolloidteilchen mittels Röntgenstrahlen," *Nachrichten von der Gesellschaft der Wissenschaften zu Gottingen*, vol. 26, pp. 98–100, 1918.

[43] G. Bayramoğlu, M. Yilmaz, A. Ü. Şenel, and M. Y. Arica, "Preparation of nanofibrous polymer grafted magnetic poly(GMA-MMA)-g-MAA beads for immobilization of trypsin via adsorption," *Biochemical Engineering Journal*, vol. 40, no. 2, pp. 262–274, 2008.

[44] D. Fischer, T. Bieber, Y. Li, H.-P. Elsässer, and T. Kissel, "A novel non-viral vector for DNA delivery based on low molecular weight, branched polyethylenimine: effect of molecular weight on transfection efficiency and cytotoxicity," *Pharmaceutical Research*, vol. 16, no. 8, pp. 1273–1279, 1999.

[45] B. Liang, M.-L. He, Z.-P. Xiao et al., "Synthesis and characterization of folate-PEG-grafted-hyperbranched-PEI for tumor-targeted gene delivery," *Biochemical and Biophysical Research Communications*, vol. 367, no. 4, pp. 874–880, 2008.

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